PROMPT PUBLICATION

The Author can greatly assist the Publishers of this Journal in attaining prompt publication of his paper by following these four suggestions:

1. *Abstract.* Send with the manuscript an Abstract containing not more than 250 words, in the precise form of The Bibliographic Service Card, so that the paper when accepted can be scheduled for a definite issue as soon as received by the Publisher from the Editor.

2. *Manuscript.* Send the Manuscript to the Editor prepared as described in the Notice to Contributors, to conform to the style of the Journal (see third page of cover).

3. *Illustrations.* Send the Illustrations in complete and finished form for engraving, drawings and photographs being protected from bending or breaking when shipped by mail or express.

4. *Proofs.* Send the Publisher early notice of any change in your address, to obviate delay. Carefully correct and mail proofs to the Editor as soon as possible after their arrival.

By assuming and meeting these responsibilities, the author avoids loss of time, correspondence that may be required to get the Abstract, Manuscript and Illustrations in proper form, and does all in his power to obtain prompt publication.

THE ANATOMICAL RECORD, VOL. 24, NO. 1
AUGUST, 1922.

Resumen por el autor, Robert Chambers.

Un nuevo aparato y métodos para la disección e inyección de las células vivas.

El autor presenta una descripción detallada de un micromanipulador mecánico basado en un nuevo principio, de simple construcción y que posee rasgos que permiten al operador mover microagujas y pipetas para la disección e inyección de las células vivas y para aislar bacterias con facilidad y precisión. El principio del aparato es un sistema de barras rígidas reunidas mediante muelles sobre los cuales actúan tornillos para separarlas. Una forma del instrumento puede sujetarse mediante pinzas directamente sobre la platina del microscopio, la otra puede fijarse sobre un pilar rígido atornillado en una base metálica sobre la cual se fija el microscopio mediante pinzas.

El autor incluye una descripción de los métodos para obtener microagujas y pipetas de vidrio, asi como la de la cámara húmeda en la cual se llevan a cabo las operaciones de disección e inyección. Discute también el empleo de condensadores de larga distancia focal. Así mismo, describe un método para obtener de un modo simple y efectivo un aparato para la microinyección, el cual se emplea con el micromanipulador. El trabajo lleva un apéndice en el que presenta una revisión histórica de los aparatos empleados en la microdisección hasta el presente. La ventaja del micromanipulador descrito en este trabajo sobre los fabricados hasta el presente es su simplicidad, su firmeza, su exactitud de movimiento y la falta total de movimientos perdidos aunque se use durante mucho tiempo.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 3

MEDIC
FEB 10 1923
LIBRARY

NEW APPARATUS AND METHODS FOR THE DISSECTION AND INJECTION OF LIVING CELLS

ROBERT CHAMBERS

Cornell University Medical College, New York City

FIVE FIGURES

INTRODUCTION

Operative work on the living cell has long been the aim of investigators in cytology and in experimental embryology. It was, however, not till Barber developed his method that any serious attempt could be made to dissect cells under magnifications high enough to enable one to observe in detail the various steps of the operation. The big feature of his method, aside from the making of needles and pipettes stiff and yet fine enough to puncture red blood corpuscles, consists in his moist chamber, which allows the needle tips to be operated in a drop hanging from a cover-slip in the chamber. This method eliminates all obstacles between the objective and the cover-slip, thereby permitting the use of the highest-powered objectives. Unfortunately, his instrument for manipulating the needles, unless very skillfully made, has too much lost motion, and wear and tear soon render the movements jerky and undependable.

Barber uses his apparatus principally for the isolation of bacteria. In 1912 Kite (Kite and Chambers, '12) applied Barber's method to cytological investigation. The difficulty of handling Barber's apparatus limited the number of investigators in this field and as the work in microdissection progressed the need of a more accurate and simple instrument became imperative.

The instrument described in this paper, a preliminary account of which has been published ('21), has the following advantages over any instrument hitherto made: a) simplicity of construction,

b) no lost motion through wear and tear, c) accurate and continuous control of the movements of the needle or pipette tip in any direction under the highest magnifications of the microscope, d) maintenance of the needle tip in one plane while it is being moved back and forth in any of the three directions, and e) existence of preliminary adjusting devices which facilitate placing the needle or pipette quickly into position.

The basic principle of the instrument consists in rigid bars which are screwed apart against springs. The movements imparted are in arcs of a circle having a radius of about two and a half inches. As the extreme range of movement of the fine adjustments is only 2 mm. (of which only one is necessary) the curvature of the arc is unnoticeable.

The movements performed by the instrument are so accurately controlled that one can readily carry out such delicate operations as puncturing mammalian blood corpuscles, tearing off the sarcolemma of a muscle fiber, drawing out nuclear chromatin strands and even cutting up the chromosomes of insect germ cells. The glass needles used for these operations taper rapidly to a point invisible under the oil immersion objective. With the micropipette, the bore of which need be no larger than one micron in diameter, one can either inject substances into or withdraw material from a cell.

For the isolation of bacteria the instrument is not only steadier than Barber's apparatus but has new features which facilitate greatly the method of procedure. Its application to bacteriological purposes is more specifically dealt with in the Journal of Infectious Diseases.

I take this opportunity of expressing my deep obligation to Mr. W. H. Farnham, mechanician in the department of Chemical Engineering in Columbia University, to whose skill and faithful workmanship the practical evolution of the instrument is due. I wish especially to acknowledge assistance from Dr. Milton J. Greenman of The Wistar Institute and Dr. C. V. Taylor of the University of California. I wish also to express my appreciation to many friends for valuable suggestions. The principle involved in the construction of the micromanipulation instrument is patented.

A MECHANICAL MICROMANIPULATOR FOR CONTROLLING THE MOVEMENTS OF A MICRONEEDLE OR MICROPIPETTE IN THE FIELD OF A COMPOUND MICROSCOPE

The principle of this device is demonstrated on considering the mechanism for the movements in one plane only (fig. 1, b). This consists of three bars of rigid metal connected at their ends to form a Z-like figure by resilient metal acting as a spring hinge.

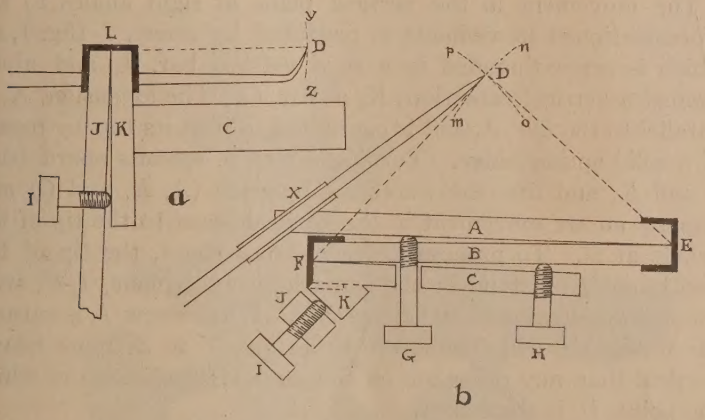


Fig. 1 Diagram showing the working principle of the micromanipulator. In 1,a, where the instrument is viewed from the side, screw, *I*, moves needle tip through vertical arc, *y–z*. In 1,b, where the instrument is viewed from above screws, *G* and *H*, move the needle tip through the horizontal arcs *m–n* and *o–p*.

By the action of certain screws the bars can be forced apart; on reversing the screws the bars return to their original position owing to the spring action at the end of the bars. By these means arc movements may be imparted to the tip of a needle when placed in the proper position.

The needle or any instrument, the tip of which is to be manipulated, is held in a carrier fastened to the free end of a bar, *A* at *X*. The needle is made to extend so that its tip is at the apex of an imaginary triangle at *D*. In order to obtain two movements at right angles to one another and in the horizontal plane the tip of the needle must be at the apex, *D*, of a right-

angled isosceles triangle the base of which is a straight line joining the centers, *E* and *F*, of the two springs holding the three bars, *A*, *B*, and *C*, together. The shank of screw, *G*, passes through a large hole in bar, *C*, and is screw-threaded in bar, *B*. Turning it spreads apart bars, *A* and *B*, and imparts an arc movement to the needle tip at *D* at right angles to that procured by turning screw, *H*.

The movement in the vertical plane at right angles to the aforementioned movements is produced by screw, *I* (fig. 1, a), which is screw-threaded in a rigid vertical bar, *J*, and abuts against a vertical extension, *K*, of bar, *C*. The extension, *K*, is parallel to the bar, *J*, and is connected to it at its top by means of a solid spring hinge. Turning screw, *I*, spreads apart bars, *J* and *K*, and lifts the whole combination (*A*, *B*, and *C*) and imparts an arc movement in the vertical plane to the tip of the needle at *D*. To procure a vertical movement, the tip of the needle at *D* must lie in the same horizontal plane, *L-D*, with the spring fastening *K* and *J* together. When screw, *I*, is turned, the needle tip will then move in an arc, *Y* to *Z*, more nearly vertical than any other arc on the same circumference of which the point, *D*, is the center.

There are two models of the micromanipulator. One is fitted with a clamping device with which it can be fastened directly to the front of the microscope stage (fig. 2; cf. fig. 3, e).[1] The other is fastened to a rigid pillar rising from a large metal base on which the microscope is clamped (fig. 3, a). The horizontal bars of the instrument extend diagonally across the corner below the level of the stage. They do not interfere with the substage accessories of the microscope nor with any of the known types of mechanical stages.

The necessity of having one or two instruments is, of course, conditioned by the type of work to be done. For picking up bacteria one is sufficient. For microdissection in experimental embryology a great deal can be done with one instrument, but for cell injection in general and for tissue cell dissection two

[1] Steadiness may be assured by a brace, one end being screwed to the rigid vertical part of the instrument and the other end to the foot of the microscope.

instruments are indispensable so that two needles or a needle and a pipette may be manipulated simultaneously. When two instruments are to be used both must be placed at the front of the microscope so that the needles may extend, side by side, into the moist chamber from the front. As the horizontal bars of each instrument extend diagonally under the microscope stage

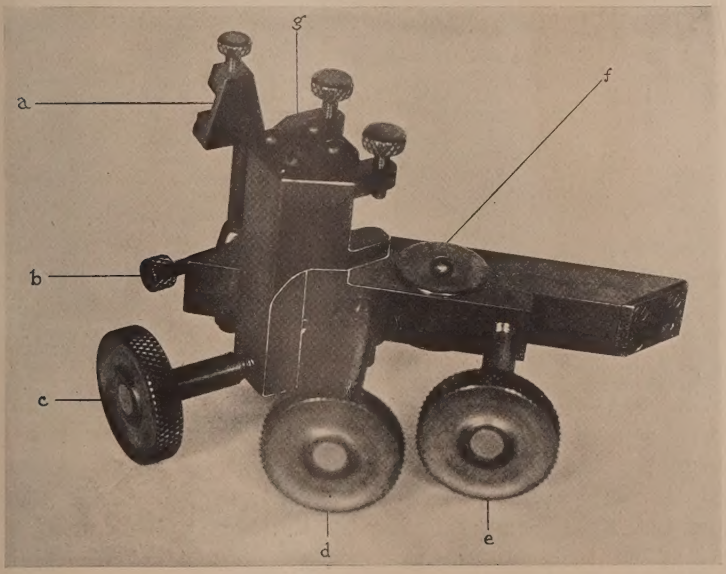


Fig. 2 Left-handed micromanipulator to be clamped to microscope stage. *a*, needle carrier with clamping screw; *b*, screw to clamp post of needle carrier; *c*, screw for up-and-down movement; *d* and *e*, screws for lateral movements; *f*, disc guide for the horizontal bars; *g*, stationary or rigid part of instrument with lugs by means of which instrument is clamped to microscope stage. Screw, *b*, clamps the coarse adjustments.

one must be a mirror image of the other. According to their position with respect to the microscope these two models have been designated as left-handed and right-handed. For bacteriological work, where it is more convenient to work from the left, the right-handed model is to be preferred as it can be swung around and fastened to the left side so that the pipette may

extend into the moist chamber from the left. For cytological work, if one desires to have only one instrument, it is advisable to secure the left-handed form and to use it as shown in figure 3, e. The mechanical stage may then be operated with the right hand and the instrument with the left. Eventually this instrument may be supplemented with a right-handed form to be clamped to the stage or attached to a pillar. When a pair of instruments is to be used the best combination is a left-handed one clamped to the microscope and a right-handed one attached to a pillar (fig. 3). This allows one to hold the tissue on which one is operating with one instrument while the microscope is being temporarily removed for renewing the pipette of the other (see page 14).

THE SETTING UP AND THE WORKING OF THE INSTRUMENT

Figure 3 shows two instruments in place ready for work. They should be as close together as possible so that the open end of the moist chamber need not be too wide to accommodate the needles. This leaves ample room on either side for the attachment of a mechanical stage on the microscope.[2]

The instrument is provided with means for a preliminary adjustment of the needle in any direction. By these means the needle tip can be quickly centered in the field of a low-powered objective and raised close to the hanging drop in which it is to operate. Before centering the tip the bars which control the fine adjustments must be put into a state of tension by giving a few turns to the milled heads of each of the three screws. The instrument is now ready for action.

The milled heads of the screws which control the lateral movements are provided with holes for rods to be used as levers. A most useful accessory is a wire-wound flexible shaft about 2 feet 6 inches long (fig. 3, c) with a milled head at one end (fig. 3, d) and the other end attached to the screw controlling the up-and-down movement. Curving the shaft around one side of the microscope brings the control of this screw, which is the one most

[2] In the case of the Bausch & Lomb and Spencer stages, it may be necessary to replace the screw clamping the front end of the stage by one with a smaller head.

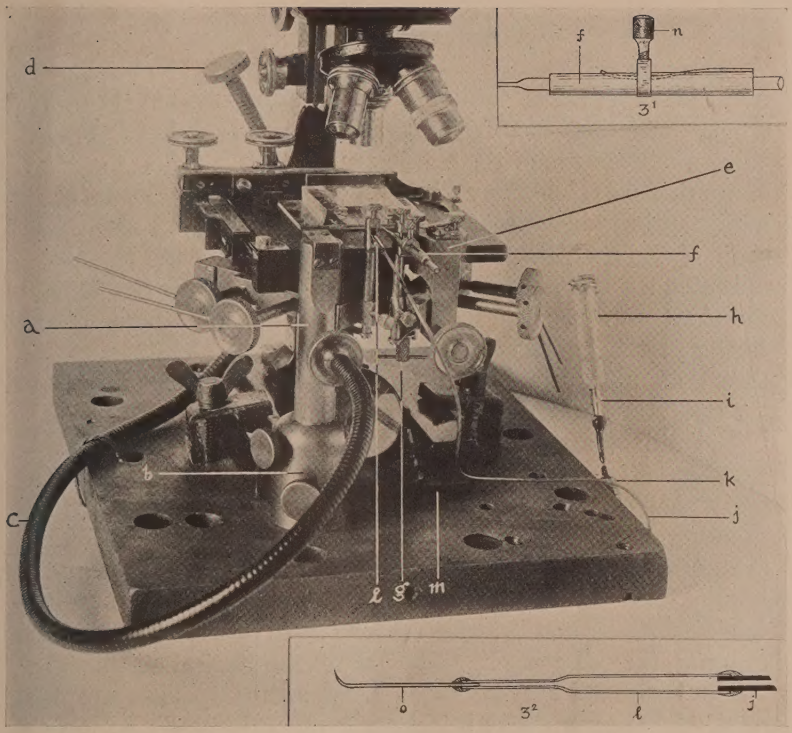


Fig. 3 Microscope with two micromanipulators and the microinjection apparatus in place. *a*, right-handed manipulator on pillar set in collar, *b*, fastened to base on which microscope is clamped; *c*, flexible shaft attached to screw for up-and-down movement with its milled head at *d*. (Note that screws for lateral movements are controlled by levers.) *e*, left-handed manipulator clamped to left front of microscope stage. In its needle carrier is clamped brass collar, *f*, within which shaft of needle slides. (See detail in fig. 3¹.) The coarse adjustment for raising and lowering needle carrier is done by screw, *g*; that for the lateral movements is done by turning the post on its axis.

Injection apparatus. *h*, Luer syringe set in its butt, *i*, cemented to curved brass tube, *j*. This is clamped to base at *k*. Its other end is cemented into glass tube, *l* (see detail in fig. 3²), clamped in needle carrier of the right-handed manipulator, *a*.

Most of the holes in the base are unnecessary. Foot of microscope is held by two screw clamps. The adjustable guide, *m*, keeps microscope in proper alignment.

Fig. 3¹ Detail of brass collar (*f* in fig. 3) which facilitates in-and-out movement of needle or pipette; *n*, screw which presses on a spring to clamp the needle in the collar.

Fig. 3² Detail of glass tube of injection apparatus (*l* in fig. 3) cemented on brass tube, *j*; *o*, shank of micropipette cemented into end of glass tube. The pipette is readily changed by softening the sealing wax which holds it.

frequently used, close to that of the fine adjustment of the microscope. The shaft also facilitates the use of both hands for the various movements of the one instrument.

Another useful accessory is a brass collar $1\frac{1}{2}$ inch long (fig. 3) with a spring which projects into its lumen through a slot. The shaft of the needle is slipped through the collar and the screw, clamping the spring, tightened sufficiently to enable one to slide the shaft evenly. The collar is then clamped into the needle carrier of the instrument. This arrangement facilitates sliding the needle into or out of the moist chamber without danger to the tip of the needle.

The micromanipulator is intended to be used with the mechanical stage of the microscope. The mechanical stage moves the moist chamber (fig. 3). As the cell or tissue to be dissected lies in a drop hanging from the roof of the chamber, the motion imparted by the mechanical stage moves the cells against the microneedle. Indeed, most of the dissection, where a single needle is used, is done by first bringing the needle tip into the cell and then dragging the cell away by means of the mechanical stage.

The horizontal movements of the micromanipulator are used mostly for the purpose of bringing the tip of the needle accurately into a desired spot in the field of the microscope preparatory to the actual operative work. In order to insure the greatest possible steadiness to the vertical movement, the part of the instrument which imparts this movement adjoins and is manipulated from the stationary and rigid part of the instrument. To make this possible the present design incorporates a theoretical error which can be understood from figure 1, a. Turning screw, *I*, to produce the vertical movement throws the combination of bars *A*, *B*, and *C*, out of the horizontal, and it is these bars upon which the lateral movements of the needle depend. However, the angle at which these bars are placed minimizes the error so that it is unnoticeable.

Guides exist in the instrument to insure a true travel of the bars as they spread apart or come together. The guide for the bar which produces the vertical movement consists of a depression in the stationary part of the instrument into which the verti-

cal bar fits. The guides of the lateral movements are two metal discs which can be tightened or loosened by screws. The upper one is seen in figure 2, f. They correct two possible errors which may occur on reversing the direction of movement, viz., a dropping of the needle or pipette out of focus and a shifting to one side.

The first error can be corrected by tightening one or both of the guides; the second, by loosening them. The guides, therefore, must be neither too tight nor too loose. The first error is the more serious of the two. It is due to an unequal tension in the springs which throws the tip of the moving screw to a different spot on the bar against which it abuts. If this be not corrected, the screw will in time wear a depression in the brass bar that is out of center thus accentuating the error. The second error is due to the guides being too tight so that they bind and prevent the bars from making a true return. If not corrected, this error will be gradually eliminated with the wear of the frictional surfaces.

By an accidental knock the horizontal bars of the instrument may be jarred out of place or the fine adjustment screws injured. If the upper and lower surfaces of the horizontal bars are not flush loosen the guide discs (fig. 2, f) also the screws of the springs on the ends of the bars and, with a wooden mallet, gently hammer the bars till they are flush. Then tighten the guide discs to keep the bars flush and carefully tighten the screws of the spring. If the screws have been bent by the accident they must be changed otherwise tightening them will again pull the bars out of place. If the guide discs are bent they also must be changed. A more serious accident is when the fine adjustment screws are injured. The steel shafts of the screws may be bent or they may have cut into the brass so as to loosen the threads. This tends to throw the shaft of the screw out of center. In such a case somewhat larger screws must be made and accurately centered opposite the bar against which it abuts.

THE SUBSTAGE CONDENSER AND THE METHOD OF MAKING BARBER'S MOIST CHAMBER AND GLASS NEEDLES

For critical illumination the height of the moist chamber must be equal to the working focal distance of the substage condenser.

The Abbe condenser can be used by removing the top lens. The focal distance of the remaining lens is almost one inch. In the Bausch and Lomb microscope the substage can easily be arranged to raise this lens sufficiently to have at least half its focal distance above the surface of the stage. This is ample, for one seldom requires a moist chamber as high as half an inch. The focal distance of this lens can be reduced and its illuminating power correspondingly increased by placing the lens of a 10X dissecting lens on top of it. This combination has a focal distance of about $\frac{3}{8}$ of an inch and, if the substage can be raised to bring the top lens flush with the upper surface of the stage, all of this distance may be used for the height of the moist chamber. Better results are secured with a triple lens condenser with its top lens removed. Such a condenser from Leitz which I am using has a working focal distance of $\frac{3}{8}$ of an inch. One may also use condensers which are made with a specially long working distance for projection apparatus, in which a cooling trough is placed between the condenser and the slide.

If the working focal distance of the condenser be less than $\frac{3}{8}$ of an inch, it is well to have two moist chambers, one for critical work and the other, from $\frac{3}{8}$ to $\frac{1}{2}$ an inch high, for ordinary work. This is advisable, because it is easier to make needles for the higher chamber.

The moist chamber is of glass (fig. 4). The base is a thin glass slide about $2\frac{3}{8}$ x 2 inches in size. The sides consist of strips of plate glass about $1\frac{7}{8}$ inches long and $\frac{1}{4}$ inch wide, and of a height determined upon by the available condenser. One end of the chamber is closed with a strip of glass of the same height as the sides and backed by another strip a fraction higher, in order to prevent a cover-slip from sliding beyond it. The trough of the chamber should be from $\frac{3}{4}$ to $\frac{7}{8}$ of an inch wide. The strips are cemented with any ordinary glass cement. Heated Canada balsam serves well. Near the closed end of the trough a small strip of glass should be cemented across the trough to provide a well for water. When cementing the long strips to the base, care must be taken to have the top surface of the strips horizontal. This may be done while the cement is still soft by

focusing on the upper surface of the strips and by manipulating the strips until all parts of their surfaces lie in one focal plane.

The well in the chamber is to be filled with water and, in order to distribute the moisture throughout the chamber, strips of blotting-paper should be placed along the sides of the trough with the inner end in the water well. One may substitute for the well strips of blotting-paper laid across the trough. This moist chamber is designed for cover-slips of a size 24 x 40 mm. The cover-slip is sealed on the chamber with vaseline. Square

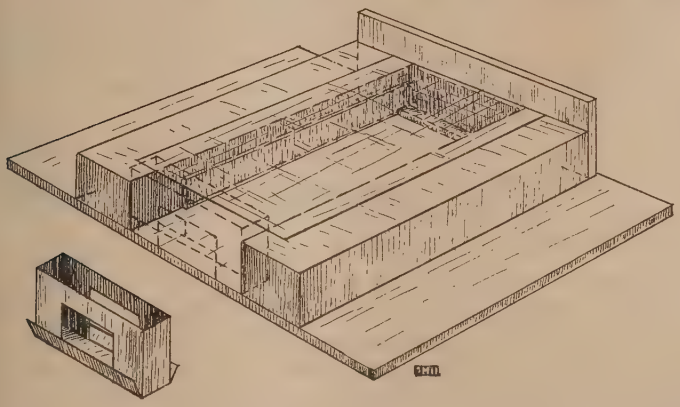


Fig. 4 Moist chamber and cardboard trough for closing open end of chamber. When the needles are in place (cf. fig. 3), the trough is placed over shanks of needles (dotted lines at open end of chamber) and filled with vaseline.

cover-slips may also be used, if the rest of the chamber be roofed with other strips of cover-glass.

The moist chamber is open at one end to permit the entrance of the microneedles or pipettes. To prevent undue evaporation, especially when a preparation is to be left over night, the open end may be temporarily closed by means of a paraffined thin cardboard trough of a shape shown in figure 4. The trough is placed over the shafts of the needles and filled with soft vaseline containing a few threads of cotton to give substance to the vaseline. The vaseline closes around the shafts of the needle and seals the opening of the chamber without interfering with the movement

of the needles. To prevent the vaseline from spreading on the floor of the moist chamber, it is well to have a shallow pan of cardboard set under the shafts of the needles for the trough to rest upon.

The hanging-drop containing the cells or tissue to be operated upon is placed on the cover-slip which is then inverted over the moist chamber.[3] To prevent the vaseline from spreading on the cover-glass and from contaminating the hanging-drop, a thin film of melted paraffin may be spread and cooled on the cover-glass bounding the area to be occupied by the hanging-drop.

The needles are made from either soft or hard glass tubing. If a brass collar is used to serve as a guide for the in-and-out movement (fig. 3), the glass tubing should be selected to fit the collar. What I use is a fraction less than $\frac{1}{8}$ inch in outside diameter. The thicker the wall of the tubing the firmer tends to be the tip of the needle made from it. The method of making the needle is given in a paper of Barber's ('14) and in one of mine ('18). A brief account will suffice here. Acetylene or ordinary illuminating gas may be used. For a microburner use a piece of hard glass tubing bent at right angles and with the burner end closed except for the smallest aperture that will retain a flame. This may be done by heating the end and pinching it with forceps. The size of the flame may be regulated by a screw pinch-cock on the rubber tube, figure 5, h.

To make the needles, proceed as follows: 1) In an ordinary burner draw out one end of a glass tube with a capillary of about 0.3 to 0.5 mm. in diameter (fig. 5, a). 2) Lower the flame of the microburner to the smallest flame possible. Now hold the shank of the tube in the left hand and grasp the capillary at its end either with the thumb and finger of the right hand or with forceps having flat tips coated with Canada balsam. Bring the capillary over the flame and pull gently till the capillary parts. The hands should remain on the table during the

[3] For placing a hanging-drop after the moist chamber has been covered, a convenient pipette is one with its end drawn out into a curved capillary and the tip bent at an angle so that, on insertion into the moist chamber, the tip will touch the undersurface of the cover-slip. With a rubber tube to reach one's mouth, a small drop is readily deposited.

process and, as the capillary parts, lift the glass away from the flame by turning the hands slightly outward. The capillary will separate with a slight tug. The tip should be like that in figure 5, c. If too little heat be used and the pull made too suddenly, the capillary may part with a snap with a broken tip.

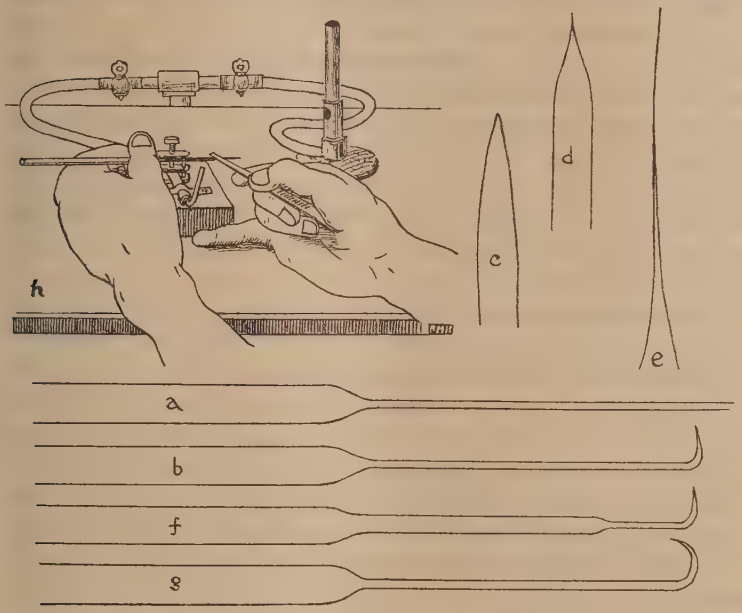


Fig. 5 Method of making the needles. *h*, position of hands when making needles over microburner; *a*, glass tube with capillary; *b*, needle with tip bent up; *c*, a good needle tip; *d*, needle tip serviceable for converting into a pipette; *e*, unserviceable tip drawn out into a hair; *f*, needle with stout shank; *g*, needle with tip bent back for cutting purposes.

If too much heat be used, the tip is drawn out into a long hair, figure 5, e. 3) Bend the capillary at right angles by heating it just back of the point and pushing up with a dissecting needle, 5, b. The length of the needle beyond the bend is conditioned by the height of the moist chamber to be used. The type of needle shown in 5, g, is used for cutting by bringing the upper limb of the needle below and up into the cell.

APPARATUS FOR INJECTION AND FOR THE WITHDRAWAL OF MATERIAL FROM A LIVING CELL

Barber's mercury pipette method, which depends upon the expansion and contraction of mercury by heat and cold, although excellent, is troublesome to make and easily broken. Taylor ('20) devised an instrument which depends upon a plunger to exert pressure on an enclosed mercury column. With mercury, however, it is difficult to maintain a plunger for any length of time without leakage. I described an apparatus ('21) in which mercury or Nujol oil is enclosed in a thin-walled steel cylinder. Pressure on the wall of the cylinder exerts the driving force necessary for injection. This works very well, but it requires special apparatus and the difficulty of securing a cylinder the walls of which are sufficiently resilient renders the apparatus somewhat unserviceable.

The apparatus shown in figure 3, does all the work of any device hitherto described and has the advantage of being extremely simple to make. All that is required is a carefully selected glass Luer syringe of about 2 cc. capacity, a piece of fine brass tubing of about 2 mm. outside diameter and two feet long (small, extra soft brass tubing used for lighting purposes is also serviceable), a metal rod 1 inch long with a hole through it large enough to receive the brass tubing, a piece of $\frac{1}{8}$-inch glass tubing, some de Khotinsky cement or ordinary sealing wax and an ordinary small horseshoe clamp.

First seal the metal butt of the Luer syringe to one end of the brass tubing. Slip the metal rod over the tubing and cement it an inch or two away from the syringe attachment. At the other end of the brass tubing seal a short piece of $\frac{1}{8}$-inch glass tubing, the free end of which has previously been drawn out into a capillary an inch or so long and about 1 mm. in inside diameter (fig. 3[2]).

When cementing the brass tube to the syringe attachment and to the glass tube, have a wire inserted far into the brass tube before applying the cement. The tip of the brass tube, from which the wire projects, is then coated with cement and the part to be cemented pulled over it. While the tube is still warm,

withdraw the wire with a gentle twirling motion. This draws the cement out around the ends of the brass tube on the inner surface of the projecting glass tube and prevents the formation of pockets in which air may be trapped. In the make-up of the entire system one must exercise care to prevent air from being trapped, for the presence of the air-bubbles vitiates the accurate control of pressure in the apparatus.

The brass tube where the metal rod encloses it is to be clamped to the foot of the microscope or to a base which is rigidly attached to the microscope, figure 3. The short end of the tube, projecting from the rod, is bent so that the syringe, when set into its butt stands more or less upright. The long end of the tube is carefully curved and bent, so that the glass tube which is sealed on the end will rest in the needle carrier of the micromanipulator and its capillary project over the stage of the microscope with its end about $1\frac{1}{2}$ inches from the field of the microscope objective.

The Luer syringe must now be charged with distilled water which has been boiled and the apparatus filled to within $\frac{1}{8}$ of an inch from the tip of the glass capillary. Before stopping, however, it is well to run water through the apparatus for some time to drive out all the air. Before charging the syringe for the last time the plunger should be coated with heavy stop-cock grease. This much of the apparatus can be kept permanently ready for use.

The micropipettes are made from microneedles drawn out of thin-walled capillary glass tubing. When finished, the shaft of the needle should be at least $1\frac{1}{2}$ inches long and large enough to fit snugly into the glass capillary of the apparatus. This can be readily done by drawing out a supply of thin-walled glass capillaries and preserving those which fit a sample the size of the capillary of the apparatus. The needle end of the shaft should be bent at an angle, the length from the knee of the bend to the tip depending upon the height of the moist chamber. The shaft of the needle near its end is now thinly coated with de Khotinsky cement or sealing wax and, while the cement is still soft, inserted into the glass tube of the apparatus. An extra coat of cement should be added over the joint to insure the seal. The apparatus is now ready for use. The tip of the needle is brought into a

hanging-drop of water or a solution to be injected and converted into a pipette by jamming the tip against the under surface of the cover-slip until it breaks off. During the process continual pressure should be exerted on the plunger of the syringe in order to prevent pieces of glass from being sucked into the pipette. Occasionally, while attempting to make the needle in the flame, a serviceable pipette results instead. When the pipette is finally in place, all or most of the air in it should be driven out.

One can readily see that the sealing of the micropipette into the apparatus must be done away from the microscope. It is in this operation that the type of micromanipulator fastened on a pillar is of advantage. The pipette has to be frequently changed, and it is very convenient to be able to release the microscope from its base by loosening its clamps and to slip it out of the way. As soon as a fresh needle has been inserted, the microscope is readily slid back into place. For this purpose the base on which the microscope rests is provided with guides to insure its true return. When exchanging a pipette, care must be taken not to clog the lumen. This can be done by using a minimum amount of cement and by having the lumen of the tube into which the shaft of the pipette is to be inserted as clean as possible.

The use of thin-walled tubing for making the micropipette is to insure having the largest bore possible at the tip of the pipette. The thickness of the wall and the size of the lumen of the glass tube tend to maintain their original proportions when drawn out in a flame. Often, however, it is more convenient to have pipettes with stouter walls. Such pipettes are less readily broken but, owing to the smaller-sized lumen, run the risk of quickly clogging. The best pipettes are made from hollow needles with a rapidly tapering tip (fig. 5, d), for needles with a long taper are apt to break anywhere.

A necessary precaution is to have the capillary from which the needle is to be made perfectly dry. The presence of the least moisture may result in alternating columns of water and air in the pipette tip which no amount of pressure will expel.

Water seems to be the best medium for transmitting pressure in the apparatus. Mercury is apt to break and allow air or

water to leak past it when it reaches the tip of the pipette. When this occurs, the separated droplet of mercury clogs the aperture. Mercury also tends to leak past the best plunger made.[4] The disadvantage of using water is the risk of its diffusion into the solution to be injected. If a considerable amount of the solution be drawn into the pipette, this risk is minimized. A good method is to color the water (e.g., with Nile-blue chlorhydrate or with neutral red). The solution drawn into the pipette from a hanging-drop is then visible by contrast. For ordinary purposes a cushion of air between the water and the injection fluid serves well.

Oil is unsuitable because, in spite of all precautions, it occasionally comes into contact with the hanging-drop containing the tissue to be operated upon; it then spreads over the surface of the drop and injures the preparation. It also dissolves de Khotinsky cement and sealing wax which are so convenient for cementing the pipette to the apparatus.

Manipulation of the syringe is facilitated by fastening it in a frame and by using a milled screw to press the plunger. I use a microscope for this purpose with the objective, substage and mirror removed. The syringe is passed through the center of the microscope stage where it is held firmly with a tight-fitting collar of cork. The lower end of the microscope tube rests on the top of the plunger so that pressure can be brought to bear on it by either the coarse or fine adjustments. There is no need of fastening the plunger to the microscope tube, because the resiliency of the water in the apparatus is sufficient to cause suction in the micropipette when the plunger is released from pressure.

APPENDIX

Barber's instrument is based on the principle of a carrier pushed along a groove by a screw at one end. By having a series of three carriers built up on one another, each traveling in a different direction, movements in any one of three dimensions may

[4] Leakage in the syringe can be avoided by placing a cushion of oil between the plunger and the mercury. This may also be done when water is used.

be imparted to a needle clamped to the top carrier. Hecker ('16) improved Barber's instrument, but added materially to the intricacy of its make up.

Other investigators that I know of who have devised instruments for micro-operative work are Schmidt ('69, '70), Birge ('82), Chabry ('87), Schouten ('05, '11), Tchahotine ('12, '21), McClendon ('07), Malone ('18), Bishop and Tharaldsen ('21).

Schmidt's instrument is one of historic interest only. I have already described it ('18). Chabry used a delicate spring device with which he could shoot the tip of a glass needle into an ovum to any desired depth. Schouten uses his for the isolation of bacteria. It consists of a pillar carrying a needle which may be mechanically raised and lowered. For the horizontal movements Schouten depends upon pushing the microscope on a base. McClendon attached an up-and-down movement to a Spencer mechanical stage. Tchahotine uses a mechanism attached to the tube of his microscope from which extends a glass needle curved in such a way as to bring its tip into the field of a low-power objective where it is brought into focus. Dissection of cells is carried out by moving the microscope tube and by pushing the cells against the needle tip by means of the mechanical stage of the microscope. Malone uses Schouten's method, but, instead of having a special pillar with a raising device, he mounts his pipette carrier on the tube of a second microscope whose adjustments serve as a means for raising and lowering the pipette. Bishop and Tharaldsen have a simple instrument based on a principle somewhat resembling mine but lacking in proper control for one of the two lateral movements. Recently I have heard that Zeiss is manufacturing a micro-dissection instrument which is a modification of Barber's apparatus with both coarse and fine adjustments.

Tchahotine and Bovie have recently devised a method for producing localized injury in a cell by means of ultra violet rays. The method is very ingenious but, of course, is rather limited in its application to micro-dissection.

BIBLIOGRAPHY

BARBER, M. A. 1904 A new method of isolating micro-organisms. Jour. Kans. Med. Soc., vol. 4, p. 487.

1911 A technic for the inoculation of bacteria and other substances and of micro-organisms into the cavity of the living cell. Jour. Inf. Dis., vol. 8, p. 348.

1914 The pipette method in the isolation of single micro-organisms and in the inoculation of substances into living cells. The Philippine Jour. Sc., Sec. B, Trop. Med., vol. 9, p. 307 (reviewed in Zeitschr. wiss. Mikr., Bd. 32, S. 82, 1915).

BISHOP AND THARALDSEN 1921 An apparatus for microdissection. Amer. Nat., vol. 55, p. 381.

CHABRY, L. 1887 Contribution a l'embryologie normal et teratologique des Ascidies simples. Jour. de l'Anat. et de Physiol., T. 25, p. 167.

CHAMBERS, R. 1918 The microvivisection method. Biol. Bull., vol. 34, p. 121.

1921 a A simple apparatus for micromanipulation under the highest magnifications of the microscope. Sc., N. S., vol. 54, p. 411.

1921 b A simple micro-injection apparatus made of steel. Sc., N. S., vol. 54, p. 552.

HECKER, F. 1916 A new model of a double pipet holder and the technic for the isolation of living organisms. Jour. Inf. Dis., vol. 19, p. 306.

KITE, G. L., AND CHAMBERS, R. 1912 Vital staining of chromosomes and the function and structure of the nucleus. Sc., N. S., vol. 36, p. 639.

MCCLENDON, J. F. 1907 Experiments on the eggs of Chaetopterus and Asterias in which the chromatin was removed. Biol. Bull., vol. 12, p. 141.

MALONE, R. H. 1918 A simple method for isolating small organisms. Jour. Path. and Bacter., vol. 22, p. 222.

SCHMIDT, H. D. 1869 and 1870 The microscopical anatomy of the human liver. New Orleans Med. Jour., vol. 22, p. 627, and vol. 23, pp. 66 and 274.

SCHOUTEN, S. L. 1905 and 1907 Reinkulturen aus einer unter dem Mikroskop isolierter Zelle. Zeitschr. wiss. Mikr., Bd. 22, S. 10; ibid., Bd. 24, S. 258.

1911 Pure cultures from a single cell isolated under the microscope. Königl. Akad. Wetensch., Amsterd, Proc. Sect. Sc., vol. 13, pt. 2, p. 840.

TAYLOR, C. V. 1920 An accurately controllable micropipette. Sc. N. S., vol. 51, p. 17.

TCHAHOTINE, S. 1912 Eine Mikrooperationsvorrichtung. Zeitschr. wiss. Mikros., Bd. 29, S. 188.

1912 Die mikroscopische Strahlenstichmethode. Biol. Centralbl., Bd. 32, S. 623.

1920 La méthode de la radio-piqûre microscopique, un moyen d'analyse en cytologie expérimentale. C. R. de l'Acad. des Sc., T. 171, p. 1237.

1921 Nouveau dispositif pour la méthode de la radio-puncture microscopique. C. R. de la Soc. de Biol., T. 85, p. 137.

Resumen por los autores, Carl Hartman y Chester H. Heuser.

Un fondo negro para la fotografía de objetos en medio liquido.

Los autores dan a conocer un método para obtener un fondo sólido de color negro en la fotografía de objeto delicados y translúcidos, colocados en agua ó solución salina. Los objetos se colocan en una vasija de tamaño apropiado y esta á su vez se sitúa sobre un trozo de vidris negro, que debe sumerjirse en agua, pues para el éxito del método es necesario evitar los espacios con aire entre el objeto y el fondo. Los objetos muy pequeños se colocarán en un vidrio de reloj de vidrio transparente y este se coloca en un vidrio de reloj negro bleno de agua.

El movimiento de la superficie del agua sobre el objeto que ha de fotografiarse se impide por medio de una placa de vidrio ó cubreobjetos que se desliza sobre la vasija que contiene al objeto cuando aquella está por completo llena de liquido. Para la fotografía se usa iluminación lateral. En la lámina que acompaña al presente trabajo se han representado ejemplos de fotografías tomadas con este método.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHORS' ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 3

A BLACK BACKGROUND FOR PHOTOGRAPHING OBJECTS IN A LIQUID MEDIUM

CARL HARTMAN AND CHESTER H. HEUSER

The University of Texas and Carnegie Laboratory of Embryology

ONE PLATE (SEVEN FIGURES)

While connected with The Wistar Institute of Anatomy, under whose auspices embryological work on the opossum was done several years ago, the writers used with success the device described below for securing solid black backgrounds in the photographing of opossum eggs. The description is here given in the belief that it may be of use to others who wish to photograph similar delicate and translucent objects in a liquid medium.

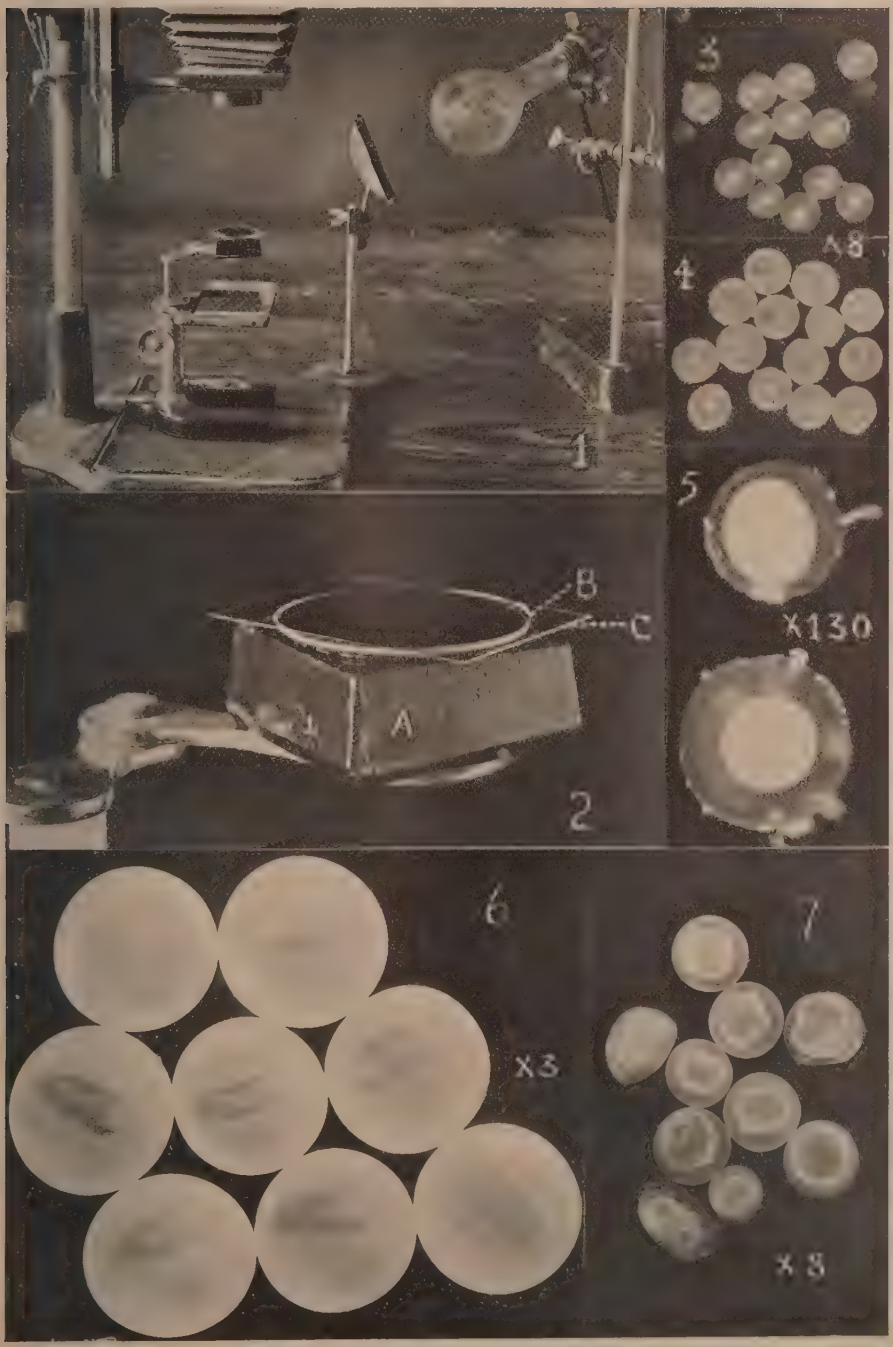
The method depends for its success upon the fact that reflecting surfaces are reduced to a minimum, air-spaces between the container and background being strictly avoided.

For photographing very small objects the arrangement shown in figure 2 was used. *A* is a dish made of black glass, known commercially as an embryological watch-glass; it has a rather deep concavity, which is filled with water. Into this is placed the shallow clear watch-glass, *B*, filled to the brim with physiological salt solution and containing the objects to be photographed. To form a stable surface the cover-glass, *C*, is slipped over the salt solution. Care should be taken to avoid all air-bubbles. The background is now a solid black. Unilateral illumination, shown in figure 1, may be used. Several samples of eggs photographed in this way are shown in figures 3 to 7.

Figure 6 reproduces a batch of embryos which were too large to photograph in the shallow watch-glass as detailed above. Hence the watch-glass was placed in a shallow Stender dish of salt solution, where, by virtue of its concavity, it functioned merely to keep the eggs together. A sheet of black glass served as a background; but here again water was necessary to dis-

place the air between the Stender dish and glass plate. This was accomplished by laying the glass plate in the bottom of a larger dish, covering it with water, and setting the Stender dish containing the specimens into it, air-bubbles being avoided as before.

The black glass plate set in water has been successfully employed by us as a black background for various objects that have to be photographed in a liquid medium.


1
3
X8
4
5
X130
B
C
A
2
6
X3
7
X3

Resumen por la autora, Ethel L. Anderson

El desarrollo de los derivados faringeos de la vaca (Bos taurus)

El presente trabajo se basa en el estudio de la faríngе de ventiocho embriones de vaca, de 3.5 á 163 mm. de longitud. Las conclusiones derivadas de este estudio son las siguientes: 1) La segunda bolsa se abre normalmente en la vaca; la cuarta no llega á to car al ectodermo. 2) La tiroides media experimenta una evolución típica. 3) El timo III, de orígen exclusivamente endodérmico se divide en una cabeza, un cordón intermedio, un segmento medio-cervical, un cordón cervico-torácico y un segmento torácico. De estas partes los cordones cervico-torácicos e intermedios presentan transformaciones tímicas tan solo en los estados avanzados. 4) Las paratiroides aparecen en forma de proliferaciones del epitelio de las bolsas, adyacente á los arcos aórticos correspondientes, y están muy vascularizadas desde sus comienzos. 5) La paratiroides III participa ligeramente en el' descenso del corazón' y no está asociada con la tiroides como una paratiroides interna, sino que permanece junto á la bifurcación de la carótida, situada medialmente á dicho vaso. 6) La paratiroides IV viene á alojarse en el aspecto medio-caudal del lóbulo lateral de la tiroides, pero nunca está incrustada en la glándula. 7) El cuerpo últimobranquial puede distinguirse todavia del material de la tiroides en el embrión de edad más avanzada de los examinados. 8) El timo IV es solamente una estructura transitoria, que no puede reconocerse después del estado de 14 mm. Solamente en dos casos de los embriones más avanzados existía un timo accesorio, el cual peuda tal vez considerarse como el' timo IV.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 3

THE DEVELOPMENT OF THE PHARYNGEAL DERIVATIVES IN THE CALF (BOS TAURUS)

ETHEL LOUISE ANDERSON

Department of Histology and Embryology, Cornell University, Ithaca, New York

ONE PLATE (FIVE FIGURES)

CONTENTS

Introduction 25
Description 26
Median thyroid 26
Complex III 27
Caudal pharyngeal complex 31
Summary 34
Literature cited 35
Explanation of figures 36

INTRODUCTION

Despite the vast amount of work on the pharyngeal derivatives in many animals, literature shows but few references to these bodies in the calf. Verdun has given a short description of the pharyngeal derivatives as found in one embryo, 160 mm. in length, together with a reference to a description of a smaller embryo by Woelfler, whose paper, however, is not accessible to the present writer. Groschuff ('96) worked with considerable accuracy on several animals, including the calf, and he has been quoted by a number of authors, among whom are Maurer and Simon. This latter author, whose chief interest was in the fate of the so-called 'lateral thyroid' (L'ebauche lateral de la glande thyroide), included in the material which he studied more than twenty calf embryos, none of which were under 20 mm. in length.

It is the purpose of this paper to present the early development of the pharynx of the calf with reference particularly to the pharyngeal derivatives. In the parathyroids especially one

can see a peculiarity in development which is apparently quite different from other forms. But brief reference will be made to the general literature; for a detailed review of the early research the reader is referred to the review by Verdun ('98) as well as to the papers of Grosser ('11), Hammar ('11), and Camp ('17).

A series of calf embryos, the property of the Department of Histology and Embryology of Cornell University, was placed at my disposal. It consisted of twenty embryos, ranging from 3.5 to 50 mm. in length and cut in transection, and the heads of eight older embryos, 58 to 163 mm. long, which were cut in sagittal section. Twelve frontal or sagittal series of younger embryos were also employed, chiefly for orientation.

The median thyroid

The development of the median thyroid is essentially that described for other mammals. The youngest embryo of the series (3.5 mm. estimated length) shows four branchial pouches, only three of which have an ectodermal contact; the fourth pouch was not observed to reach the ectoderm in any of the embryos which were examined. The 'median thyroid' appears in this embryo as a hollow bud from the floor of the pharynx, in the region just posterior to the first pouch. With the rupture of the 'thyreoglossal duct' (8.5 mm. embryo) the thyroid is left as a short solid cord or plate of cells, morphologically, as at the time of its origin, cephalodorsal to the truncus aorticus. A slight depression on the floor of the pharynx, the foramen caecum, marks the upper extremity of the duct and the point of origin of the gland. The 8.5-mm. embryo was the only one in which the foramen caecum was evident. The lower extremity of the duct, the 'ductus thyreoideus,' extends about 0.12 mm. above the body of the gland.

At about this age (8 to 11 mm.) the lateral expansion of the thyroid is dependent apparently on the growth tensions in the individual embryo, and presents considerable variation. But in every case it has maintained its position on the cephalodorsal side of the truncus aorticus. The ductus thyreoideus can be

traced forward sometimes as much as 0.3 mm., depending on the length of the downward shifting and somewhat on the angle of the section. Though the base of the 'duct' is moderately thick, the upper part dwindles down to no more than two or three cells to the section.

In their expansion the lateral lobes come into close contact, topographically, with the derivatives of the caudal pharyngeal complex—the ultimobranchial body and parathyroid IV. In the 14-mm. embryo the ultimobranchial body lies medial to and close up against the lateral lobe of the thyroid, and in the early stages, when the ultimobranchial body is large, it constitutes nearly the entire lateral lobe, while the thyroid itself is represented by slender cords or plates of cells, loosely strung together by connective tissue, as shown in figures 2 and 4; the median portion of the thyroid is more compact. Parathyroid IV lies median to the dorsal aspect of the lateral lobe, entirely outside of the parenchyma of the gland. As the embryo grows the parathyroid and the ultimobranchial body sink lower in the lateral lobe and the thyroid material comes to nearly surround the latter.

Though in earlier embryos the derivatives of the caudal pharyngeal complex lie in or near the cephalic end of the lateral lobe, in those of 30 mm. and over they are found well down within the body of the lobe; parathyroid IV is sometimes visible even after the sections have passed below the level of the thyroid itself. This shifting in position is due partly to the upward expansion and growth of the lateral lobe and partly to the continued 'descent' of the other bodies. In the 50-mm. embryo the lateral lobes contain the characteristic dark patches of cells (cf. fig. 3), the cords of the ultimobranchial body. There is, as yet, no loosening of the cells of the thyroid cords to form vesicles and no secretion of colloid until the embryo has reached 140 mm. in length. Simon ('96) also described the appearance of colloid at this time.

Complex III

In the calf embryos one finds four well-formed pouches. Only the first three of these were ever observed to establish an ecto-

dermal contact; the fourth pouch was never observed to reach the ectoderm in the series of embryos which was studied. The 8-mm. embryo showed the rupture of the membrane separating the second pouch from the exterior; this condition is quite common in calf embryos (and occasionally observed in human); in some instances it may be due to rough handling, but as the rupture was observed in several embryos which were well preserved and showed good morphological relations it was concluded to be a normal occurrence. The first of the aortic arches—which form on the anterior aspect of each pouch—has already broken its connection with the dorsal aorta in the 3.5-mm. embryo; the second, though small, is still intact; the third is large and fills most of the space in the third branchial arch, and the fourth and sixth aortic arches are not yet formed. In the 8-mm. embryo the forward growth of the anterior pouches has broken the second as well as the first arch; the remaining three arches (third, fourth, and sixth) are well formed.

The 8.5-mm. embryo is the youngest in which there is to be noted from the medial border of the third pouch the ventral elongation which is to form the entodermal thymus. As the embryo develops (9.5 to 10 mm.) thymus III becomes elongated, extending down from the pouch, directed ventrally and medially and pointed toward the truncus aorticus. In the more cephalic sections it is seen in the form of a hollow tube, dorsal and a little external to the third aortic arch (the primitive carotid). It follows the line of the carotid, swinging to the lateral and finally to the ventral side of that vessel.

When the embryo is 14 mm. in length the thymus and parathyroid III have lost their connection with the pharynx. The head of the thymus is lodged in the caudal and dorsal part of parathyroid III and the gland extends back from this as a slender cord with the lumen nearly obliterated, and follows roughly, as before, the line of the carotid. In the thoracic region the two sides of the thymus make a rather marked medial turn and come to lie over the pericardial cavity, a little to the left of the midline. Here the walls of the thymus become enlarged and lobulated; the branching cords from either side are apparently fused along their region of contact.

In connection with the development of the thymus it may be stated that a cervical vesicle is formed essentially as in man and possesses the same relations to the ganglion nodosum. It forms no part of the thymus, as it does in the pig.

In the 30-mm. embryo the division of the thymic cord and body into segments is definitely noted for the first time. The head of the thymus, slightly enlarged, lies, as before, in connection with the lower pole of parathyroid III and the whole group has shifted a little to the median side of the carotid artery, on its dorsal aspect. From the head extends a thin cord of cells, called by Badertscher ('15, who adopted the terminology of Kastschenko, '87) the 'intermediary cord,' which connects the thymic head with the 'mid-cervical segment.' In the region of the thyroid the 'intermediary cord' is very small and lies ventral to, and a little anterior to the brachiocephalic vein which is formed by the union of the subclavian and jugular veins. Below this, in the region of the truncus proper and also ventral to the superior vena cava, the thymic cord diminishes markedly in caliber to form the 'cervicothoracic cord' which connects the 'midcervical' with the 'thoracic segment.' The thoracic segment lies in the upper section of the thorax, is expanded in contact with the pericardium, and is markedly lobulated.

The above description will hold, in the main, until the 80-mm. stage is reached. Only the following differences need be noted. In the 39-mm. embryo there is no enlargement in the midcervical region. The 40-mm. stage shows a shifting of parathyroid III so that it (and consequently the head of the thymus) lies below the bifurcation of the carotid and well medial to that vessel.

After the 80-mm. stage the head of the thymus becomes markedly enlarged and lengthened. At 110 mm. the intermediary cord begins to enlarge, and at 130 mm. shows slight lobulation. Complete older series would undoubtedly show a transformation of the entire thymic cord as in the pig (Badertscher).

The dorsal element of complex III, parathyroid III, appears for the first time in the 9.5 to 10-mm. embryo in which the thymus has already elongated. It lies on the anterior and dorsal surface of the third pouch, and extends well from the pharynx

to the free margin of the pouch. Histologically, it is composed, from its earliest appearance, of cords of epithelial cells, proliferated by the entoderm of the pouch and associated from the start with a rich network of fine capillaries.

The question of the vascularization of the parathyroids is an important one and involves possibly the question of the cause of their appearance. In the human embryo they appear as rather thick, but definitely limited proliferations of epithelium on the dorsal side of the third and fourth pouches, and it is not until late in their history that they are broken by vascular channels. In the calf, on the other hand, blood vessels appear along the anterior side of the third pouch before the epithelial proliferation is even noted (9 mm. Sag.) and, after the proliferation, there is a subepithelial plexus of blood vessels not only between the cords of cells, but also to the outside of the gland. The connection of this vascular network appears to be with the anterior side of the third aortic arch, and with the dorsal side of the external carotid, going to parathyroid III on either side of the common carotid; a few of the younger embryos show direct vascular connections between the posterior side of the carotid and the parathyroid (fig. 5), and still others show combinations of these sources. In all cases the third aortic arch lies along the anterior and dorsal surface of the third pouch and is unquestionbly the source of some of the blood for the parathyroid.

In the early embryos parathyroid III lies dorsal to the common carotid artery, between it and the anterior surface of the pouch, and the surface of the parathyroid is slightly concave to conform to the shape of the carotid about which it fits. Topographically it is anterior to the bifurcation of the carotid.

In the 14-mm. embryo, in which the derivatives have broken from the pharynx, the parathyroid has the shape and position which characterize it for a number of stages; it is in the form of a thick crescent, slightly elongated in the vertical plane. On its anterior side it is concave toward the carotid and forms a cap around that vessel, just cephalad of the bifurcation of the carotid. It lies a trifle lower than the carotid body, which is seen here for the first time. The horns of the crescentic parathyroid seem

to be shaped about the sides of the carotid by the hypoglossal nerve (XII) on the exterior, and the superior laryngeal nerve on the interior surface. In the younger embryos the hypoglossal nerve lay entirely caudal and ventral to the third complex, lateral to the fourth complex and its aortic arch. A slight descent of the head of the third complex together with the forward growth the more lateral material is responsible for the altered relations. The head of the thymus is lodged in the lower pole of parathyroid III.

Subsequent stages (30 mm. and up) see a backward shifting of parathyroid III, so that most of its body now lies caudal to the bifurcation of the carotid and hence posterior to the carotid body. It does not, however, come into contact with the lobes of the thyroid as does the other parathyroid. In its shifting it comes to lie well medial to the carotid and offers a flat or slightly concave surface to that vessel. Numerous small blood vessels are seen which enter parathyroid III from the carotid both directly (fig. 5) and out through the carotid body.

The caudal pharyngeal complex

The caudal pharyngeal complex—to include under a common heading, the derivatives parathyroid IV, the ultimobranchial body, and thymus IV, of the more caudal pouches—has been a frequent source of discussion among writers, both from the standpoint of origin and from the standpoint of fate. Especially is this true of the ultimobranchial body (the 'lateral thyroid,' 'post-branchial body,' 'suprapericardial body' of other writers).

Briefly, the morphology of the fourth or caudal pharyngeal complex as it appears in successive stages in the calf embryos of the series studied is as follows:

The fourth pouch, as stated above, does not reach the ectoderm, but lies in the posterior region of the pharynx caudal to the third pouch and, because of its tardy development, is smaller than and median to the wings of the third pouch. In the 8.5-mm. embryo a long posterior and a short anterior diverticulum are evident on the ventral side of the complex and the fourth aortic arch forms to the lateral side of the pouch. The anterior

diverticulum elongates (9.5 to 10 mm.) to form the so-called 'thymus IV' with its axis directed toward the truncus as in the case of thymus III. The posterior diverticulum forms the ultimobranchial body, and in the angle between it and thymus IV, on the lateral side of the pouch in the region toward the fourth aortic arch is parathyroid IV. Like parathyroid III, parathyroid IV is from the first an epithelial proliferation from the wall of the pouch and is supplied with a rich subepithelial network of blood channels. But here it has been impossible to determine any definite and constant connection of the vascular network. In the 11-mm. embryo there is one direct blood vessel from the posterior side of the fourth aortic arch to parathyroid IV. The ultimobranchial body and thymus IV have a common opening into the pharynx, but their relative size soon changes so that thymus IV (14.5 mm.) appears to be an appendage of the ultimobranchial body; parathyroid IV, a vascularized proliferation, lies between them on the dorsolateral aspect of the pouch.

The ductus pharyngeo-branchialis IV, which early connects the complex with the pharynx, has ruptured and entirely disappeared in the 14-mm. embryo; thymus IV, nicely shown in sagittal section in the 14-mm. stage, has disappeared as such, and of the complex there only remain parathyroid IV and the ultimobranchial body. These come to lie close to the upper pole of the lateral lobes of the thyroid as that gland develops. Parathyroid IV is the more dorsal and medial; the ultimobranchial body is fast against the inner surface of the lateral lobe, but separated from the thyroid by a distinct connective-tissue sheath. A cord of cells connects the anterior side of the ultimobranchial with the parathyroid, and in the parathyroid, at the head of this cord, is a cavity—the remains of the cavity of the fourth pouch.

Even at 15 mm. the ultimobranchial body shows, in its scalloped edge, the tendency to 'bud,' which has led some authors to the belief that this, the 'lateral thyroid,' after its inclusion in the lateral lobe of the thyroid, is transformed into a part of the thyroid gland.

While the relative size and position of parathyroid IV and the ultimobranchial body vary in embryos 20 mm. to 50 mm. in

length, the same general relations hold. Parathyroid IV is round or oval in form, always situated in the dorsomedial aspect of the lateral lobe of the thyroid, sometimes above and sometimes below the level of that gland. Often, especially in the later stages, the parathyroid will lie low on the lateral lobe. Some of the blood enters it directly from the dorsal side of the carotid artery (24 mm.), while in other cases (40 mm., 50 mm.) the parathyroid is supplied with a branch from the accessory thyroid artery. It always contains, on its ventral side, a cavity from which a solid cord of cells runs forward to join with the ultimobranchial body (fig. 4). This latter structure is embedded more or less intimately in the lateral lobe of the thyroid and shows in the early stages a heavy wall and a narrow, cresent-shaped cavity. About it the cords of the thyroid tend to be thin and separated by connective tissue; below the level of the ultimobranchial body the material of the thyroid is more closely packed. Gradually the thick wall of the ultimobranchial body loosens and sends out buds which shape themselves into narrow cords or plates of cells, intermingling with those of the thyroid proper. Though in form resembling the material of the thyroid, histologically they are quite different. The nuclei of the epithelium of the thyroid are of varying size and shape and contain numerous small bits of chromatin, peripherally placed. The nuclei of the cells of the ultimobranchial body, on the other hand, are smaller, rounder, more nearly of a size, and more closely packed; they contain two or three large nucleoli, centrally located. Even in the 50-mm. embryo, in which the sections are rather thick, the material of the ultimobranchial bodies can be easily distinguished in the midst of the substance of the thyroid by its darker staining qualities. Figure 3, of a 33-mm. embryo, may be compared.

From this time on, with the marked increase in the size of the thyroid, the ultimobranchial body becomes relatively smaller and smaller and shows as thick branching cords of darkly staining cells at the dorsal side of the lateral lobe of the thyroid and at the 'hilus,' around the entrance of the blood vessels. These cords of cells branch out, but do not join with the parenchyma of the thyroid.

In the 40-mm. embryo, in connection with the lower pole of the parathyroid, is found an instance of an accessory thymus (IV). Its appearance is that of the thymus (III) and its relation to the posterior pharyngeal complex would suggest that it represents a thymus IV. A calf of 130-mm. length shows a similar structure.

SUMMARY

The following points have been noted in the morphology of the pharyngeal derivatives in the calf:

1. The median thyroid undergoes a typical evolution.
2. Thymus III, of entodermal origin only, divides into head, intermediary cord, midcervical segment, cervicothoracic cord, and thoracic segment.
3. Of these, the intermediary and cervicothoracic cords show thymic transformation only in the older stages.
4. The parathyroids appear as proliferations of the epithelium of the pouches, adjacent to the corresponding aortic arches, and are highly vascular from the first.
5. Parathyroid III is but little involved in the descent of the heart; it is not associated with the thyroid as an external parathyroid, but remains near the bifurcation of the carotid and medial to that vessel.
6. Parathyroid IV becomes lodged on the mediocaudal aspect of the lateral lobe of the thyroid, but is never embedded in that gland. It is connected, in all the embryos examined, by a cord of cells to the ultimobranchial body.
7. The ultimobranchial body can still be clearly distinguished from the material of the thyroid in the oldest embryo examined.
8. 'Thymus IV' is but a transitory structure, not recognizable after the 14-mm. stage.
9. In only two cases in the older embryos was an accessory thymus noted which might possibly be considered as 'thymus IV.'
10. From the evidence it may be concluded that: *a*) The second pouch normally opens in the calf; *b*) the fourth pouch fails to reach the ectoderm.

I wish to take this opportunity to thank Dr. B. F. Kingsbury for the material he has put at my disposal and for helpful criticisms and suggestions. I am also indebted to Prof. S. H. Gage for the material which he has loaned to me.

LITERATURE CITED

BADERTACHER, J. A. 1915 a The development of the thymus in the pig. I. Morphogenesis. Am. Jour. Anat., vol. 17, no. 3.
1915 b The development of the thymus in the pig. II. Histogenesis. Am. Jour. Anat., vol. 17, no. 4.
1918 The fate of the ultimobranchial bodies in the pig (Sus scrofa). Am. Jour. Anat., vol. 23.

CAMP, W. E. 1917 The development of the suprapericardial (postbranchial, ultimobranchial) body in Squalus acanthias. Jour. Morph., vol. 28, no. 2.

GROSCHUFF, K. 1896 Bemerkungen um der vorläufigen Mitteilung von Jacoby; Ueber die Entwickelung der Nebendrüsen der Schilddrüse. Anat. Anz., Bd. 12, no. 21.

GROSSER, H. 1911 Zur Entwicklung des Vorderdarmes menschlicher Embryonen bis 5 mm. grösster Länge. Sitzungsber. d. Kaiserl. Akad. d. Wiss. Wien. Math.-Naturf. Kl., Bd. 120; Abt. III.

HAMMAR, AUG. 1911 Zur groberen Morphologie und Morphogenie der Menschenthymus. Anat. Hefte, Bd. 43, H. 1.

KASTSCHENKO, N. 1887 Das Schicksal der embryonalen Schlundspalten bei Säugetieren. Arch. f. mikr. Anat., Bd. 30.

KINGSBURY, B. F. 1915 The development of the human pharynx. I. The pharyngeal derivatives. Am. Jour. Anat., vol. 18, no. 3.
1914 On the so-called ultimobranchial body of the mammalian embryo; man. Anat. Anz., Bd. 47, no. 24.

MAURER, F. 1906 Die Entwicklung des Darmsystems in Handbuch d. Entwges. der Wirbeltiere. Edited by O. Hertwig, Bd. 2, s. 108–252.

PRENANT, A. 1900 Contribution à l'étude du développement organique et histologique du thymus, de la glande thyroide, et de la glande carotidienne. La Cellule. T. 10.

SANDSTRÖM, J. 1880 Om em neg Koertel hös menniskan och åtskilliga doggdjur. Upsale Läkerefoerenings Foerhandlinger, 15.

SIMON, C. 1896 Thyroïde latérale et glandule thyroidienne chez les mammifères. Thèse, Nancy.

VERDUN, P. 1898 Dérivés branchiaux. Thèse, Toulouse.

WOELFLER 1880 Ueber die Entwickelung und den Bau der Schilddrüse. Berlin.

PLATE 1

EXPLANATION OF FIGURES

1 Section through the pharynx, showing the third branchial pouch and the formation of parathyroid III on the anterior and (dorsal) side of the pouch. Section also shows direct vascular connection from the posterior side of the third aortic arch to the anterior surface of the corresponding pouch. The second pouch is open. Embryo calf, 11.5 mm. Photograph, × 33⅓.

2 Section through the caudal pharyngeal complex, to show the characteristic relations. Parathyroid IV, on the right, shows the characteristic cavity at the head of the cord which connects the parathyroid with the ultimobranchial. Embryo calf, 14 mm. Photograph, × 33⅓.

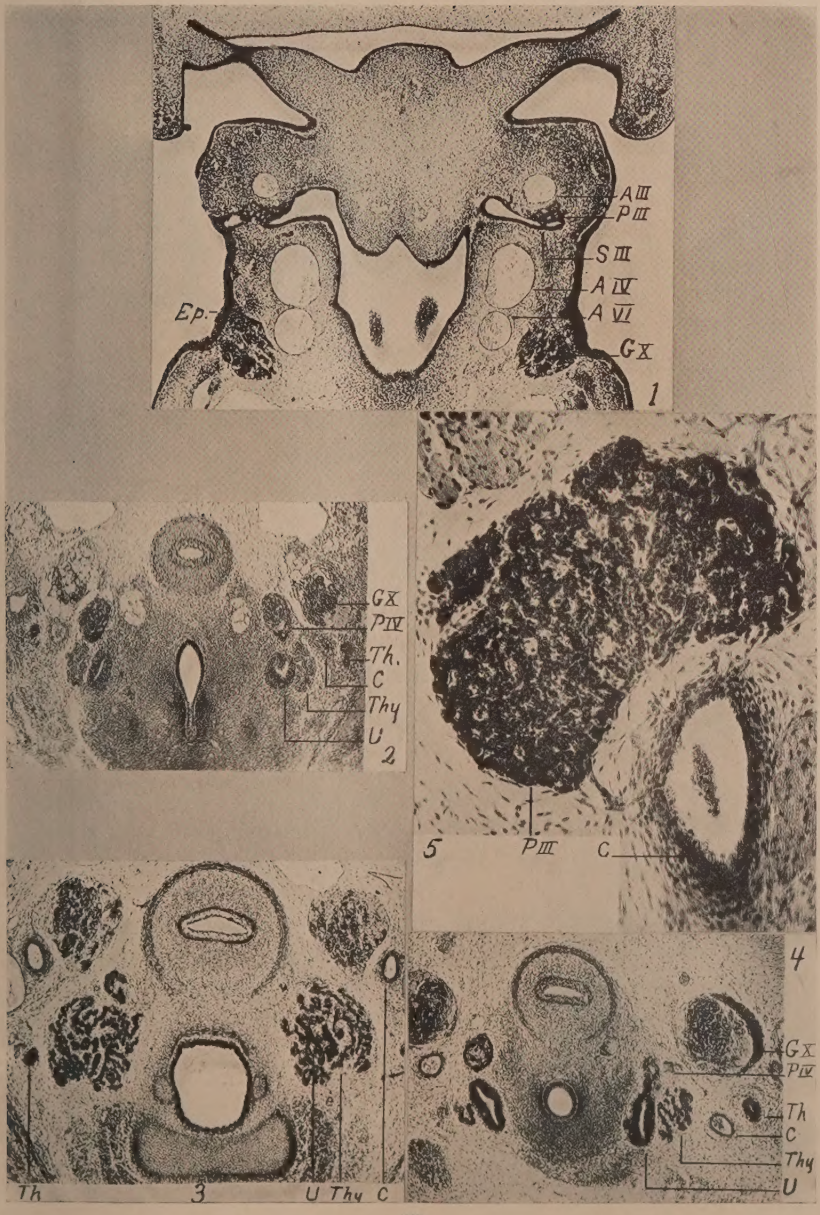
3 Section through the lateral lobe of the thyroid in a more advanced stage. The cords of the ultimobranchial body are clearly distinguishable. Embryo calf, 33 mm. in length. Photograph, × 33⅓.

4 A section through the caudal pharyngeal complex, to show the connection of the ultimobranchial body to the parathyroid IV by a cell cord. Compare with figure 2. Calf embryo of 20-mm. length. Photograph, × 33⅓.

5 Section through parathyroid III and the carotid artery, to show the direct vascular connections between the two. Embryo calf, 30-mm. length. Photograph, × 125.

ABBREVIATIONS

A. III, third aortic arch
A. IV, fourth aortic arch
A. VI, sixth aortic arch
C., carotid artery
Ep., epibranchial placode
G. X., ganglion nodosum (X)
P. III, parathyroid III
P. IV, parathyroid IV
S. III, sacculus branchialis III (pouch III)
T. A., truncus aorticus
Th., thymus
Thy., thyroid
U., corpus ultimobranchiale


AIII
PIII
SIII
AIV
AVI
Ep.
GX
1
GX
PIV
Th.
C
Thy
U
2
5
PIII
C
4
GX
PIV
Th
C
Thy
U
Th
3
U Thy C